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NUCLEAR PHENOMENA OF SEXUAL REPRODUCTION IN FUNGI[1]

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IN the light of recent researches the old dogma that the parasitic mode of life tends to the disappearance of sexuality has practically disappeared at least so far as the fungi are concerned. The evidence is now generally accepted that either a typical conjugation of normally differentiated gametes and their nuclei or some form of substitute for it is everywhere present.

Ernst and Schmidt on the basis of their studies on the root parasite *Rafflesia* have also recently emphasized the fact that there is no evidence in the case of seed plants that the parasitic habit tends to the disappearance of sexuality. Farmer and Digby have described a most remarkable substitute fusion in an apogamous fern and a still more striking case of substitute cell and nuclear fusions in another apogamous fern has been recently discovered in my own laboratory. It is proper under these conditions to examine anew and most critically all cases of cell fusions with reference to their accompanying nuclear phenomena and possible significance in the life cycle in question.

The study of sexuality in the fungi has also brought to light some fundamental modifications of the process of sexual fusion as found elsewhere which enlarge our conception of the nature and significance of gametic unions. It has also revealed most curious and striking substitutions for sexual fusions. I shall review briefly the most characteristic and significant of these variations from the ordinary methods of sexual reproduction. The fusion of the amœboid swarmspores to form the plasmodium of the slime moulds has never been satisfactorily accounted for, nor related to the processes either vegetative or sexual in other groups of fungi. With the increase of our knowledge of the possible variations which the sexual process may undergo it is becoming more possible to accept the conception that in some way the formation of the plasmodium may represent either an incipient or an aberrant type of gametic union in which the normal fusion of two gametes is replaced by a massing of indefinitely numerous cells.

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[1] A paper read by invitation before the Botanical Society of America, Boston, December 30, 1909.

The cytological study of the group is only just beginning and doubtless much is to be corrected in the fragmentary observations already published. Olive, Jahn and Kraenzlein, however, agree that there are evidences of nuclear fusions either just before spore formation or earlier in the formation of the fruit bodies, and that these karyogamies are followed by synapsis and reduction divisions. The figures given show that these forms have typically developed nuclei which are favorable for fixing and staining, and indicate that whatever disagreement exists as to what occurs in the few types studied may be expected to be cleared up by further work.

The older authors were loath to regard the union of the amœbæ to form a plasmodium as involving anything of a sexual nature, but it is quite possible that we may have to extend our conception of sexual fusions at least in their primitive forms to include cases of multiple cell fusions followed by vegetative growth, and finally the fusion of the nuclei in pairs. That cell and nuclear fusions may be thus widely separated is plain from the conditions in the rusts, and in the slime moulds, as in the rusts, nuclear fusion is followed shortly by the reduction divisions.

We can not yet regard the nuclear phenomena in the slime moulds as sufficiently cleared up. In the light of what has been found in other fungi there is however certainly no ground for believing that the nuclear history in the plasmodium is as simple as the older students believed.

As directly suggestive of what may be expected in the slime moulds, we have the well-established fact of the fusing of multinucleated gametes and the subsequent pairing of all or approximately all their nuclei. Leger and others have seen that the gametes of *Sporodinia* and other zygomycetes are multinucleated, but the accounts *of the subsequent processes in the zygospore can not* be accepted as well founded and no one yet has been able to trace the history of these minute nuclear granules.

Stevens, however, has established the entrance of many male nuclei and their subsequent pairing with the numerous nuclei of the eggs of *Cystopus bliti* and *portulacæ* and unpublished observations made in my own laboratory have confirmed his results for *C. bliti.* I have also found such multiple fusions of about 200 nuclear pairs in the large oogonia of *Pyronema.* . Claussen in a preliminary paper, while confirming the existence of the fusion of multinucleated gametes and subsequent pairing of the sexual nuclei in *Pyronema,* claims that the fusion of the pairs is not completed till they reach the ascus.

The large size of the nuclei in the ascogenous hyphæ in Claussen's preliminary figures seems to me, however, to confirm my own conclusion that the fusion is already completed at this stage. These multinucleated gametes prove convincingly that our conception of the egg and male cell must be extended to include multinucleated as well as uninucleated types. The cell at the moment of sexual union may be multinucleated as well as in its ordinary vegetative stages in the hyphæ, etc. That the nuclei fuse in pairs and not in larger numbers is further convincing evidence that they are the real bearers of the idioplasm and that the constancy of the chromosome number must be maintained by the ordinary type of doubling and subsequent reduction.

With the demonstration of the existence of coenocytic gametes the nature of the coenocyte itself becomes still clearer, as I have elsewhere pointed out. The continuous plasma membrane enclosing a coenocyte is plainly in its relation to the other cell contents to be compared with the same structure in an uninucleated cell rather than with the aggregate of membranes which bound off a mass of tissue from its environment and the cells of the tissue from each other.

It is further most interesting to note that De Bary's conception of the male element in the oomycetes as gonoplasm, a mere unbounded portion of the contents of the antheridium, has been entirely confirmed by subsequent cytological research, and it is further proof of the superior significance of the nucleus as the carrier of the idioplasm in sexual fusions that in such forms as *Cystopus candidus,* for example, it is merely one of the several nuclei in the antheridium, and that with no definitely limited cytoplasmic unit which migrates through the conjugation tube and fertilizes the egg. The differentiation of the male gamete is not here an ordinary process of cell division, but a mere flowing out of one of the nuclei of the coenocytic antheridium.

The most striking discovery as to fusion in the fungi and the one which preceded and led the way to very many of the more important later results was the observation by Wager of paired nuclei and the subsequent fusion of these nuclei in the young basidium.

Wager was mistaken in describing a series of such fusions by pairs, but the clear account of the nuclear structures which he gave and the evidence that nuclear division occurs by a karyokinesis like that of other plants and animals showed for the first time the possibility of determining the nature of the various reproductive bodies in fungi by a more exact cytological investigation of them than had before been thought possible.

Wager brought the first proof of the existence of an endokaryogamy—the fusion of nuclei not derived from separate and independent gametes as in ordinary fertilizations, but having had a similar if not identical history in the cells from which the basidium arose. Such a process was entirely unknown before in either plants or animals.

Subsequent investigations by Dangeard, Maire, Ruhland, Nichols, myself and others have shown that in the Basidiomycetes a long series of binucleated cells precedes the formation of the basidium and the nuclear fusion in it may well have something of the value of a union of differentiated gametic nuclei.

The origin of the binucleated cells in the Basidiomycetes does not apparently result from a cell fusion at a definite point in the life cycle, as is the case in the æcidium of the rusts. Such a fusion might perhaps be expected to be effected at the origin of the carpophore, but it has been established that binucleated cells may be present in the mycelium prior to the formation of the carpophore. Series of such cells may extend back almost if not quite to the germination of the spore from which the mycelium arose.

A fusion of gametes morphologically equivalent to those of other algæ and fungi seems to have quite disappeared from the group so far as known at present, though it is to be remembered that comparatively few forms have been investigated as to the early stages of the formation of the carpophore. It seems probable that the endokarygamy in the basidium may have functionally replaced an older fusion of differentiated sex cells.

We have then on the present evidence a large group (over 9,000 species, according to Saccardo) of saprophytic and parasitic organisms in which normal sexual reproduction has been replaced by the fusion of nuclei of probably separate descent through a long series of cells, but which are contained at each stage within the limits of a single cell body.

The most thoroughly worked out modification of sexual reproduction in the fungi is the fusion of uninucleated gametes producing binucleated series of cells in which nuclear fusion follows only at a much later period. Such fusions seem probably to be present in all rusts which have an æcidium or primary uredo and may occur in two forms involving in the one case merely the migration of a nucleus into a so-called fertile cell which then develops into the chain of æcidiospores, while in the other case there may be a complete cytoplasmic union of equivalent cells the so formed binucleated fusion cell then developing into a row of æcidiospores or a series of primary uredospores.

Dangeard and Sappin Trouffy discovered that the binucleated cells in the rusts originate with the æcidium, but the real significance of the binucleated condition and the method of its origin first became clear with the work of Blackman, Christman and Olive.

It is quite probable, as Blackman maintains, that these fusing cells in the æcidium of the rusts have been much modified from the ancestral conditions of the sex cells of the group, so that their fusion is to be properly characterized as a vegetative fertilization. Still there can be no question that functionally these are sexual unions, and we can hardly imagine anything more illuminating as to the relations of the male and female pronuclei in the cells produced by fusion and hence containing the double chromosome number. There can be no question here that the male and female chromosomes maintain their independence throughout the entire sporophytic life cycle and that nuclear fusion and synapsis are two closely associated phases of that more intimate union of the chromosomes which the behavior of hybrids suggests must occur at the close of the sporophyte. Such a series of binucleated cells is unknown among animals but the tendency to persistent independence shown by the pronuclei in the embryonic development of *Cyclops* shows that there is every reason for believing that here and probably in all nuclei with $2n$ chromosomes the hereditary idioplasms from the male and female parents maintain quite the same physical independence throughout the sporophyte as is so convincingly shown in the rusts.

The phenomena of conjugate division also indicate very clearly how the mechanism of karyokinesis, including centrosomes, asters and spindles, may be partly or wholly combined in a single system without necessitating a similar union of the idioplasmic units.

One of the most important points in this sexual process in the rusts is that the nuclei do finally fuse in the teleutospore. One might suppose that since the nuclei can function as physically independent units through the whole life of the sporophyte with its manifold vegetative and reproductive phases, a reduction division might be accomplished by the mere insertion of a wall between the conjugate nuclei. This may be what occurs in *Endophyllum* according to Maire. Still the fact that the nuclei fuse before the heterotypic division in practically all other rusts is certainly strongly suggestive that synapsis and its accompanying phases represent a stage of mutual influence if not of interchange of physical material between the chromosomes much more intimate than any which has preceded it in the life of the sporophyte.

On the other hand, it is plain that the nuclear fusion is unnecessary so far as the sporophyte itself is concerned. The sporophyte of the rusts, as in other plants, is the distinctively dominant and progressive phase in the life cycle of the fungus. The uredo mycelium and the rapid succession of crops of uredospores with all their adaptations for rapid spread and virulent development are a parallel in every respect to the sporophyte in every type of plant in which it is found from the ferns to the seed plants. It is plain then that the vigor and adaptability of the sporophyte are not dependent upon the combination of the sets of parental chromosomes in a single nucleus. The same results are possible with two more or less independent nuclei in the cell, each containing the chromosomes from one parent.

For the ascomycetes Dangeard extended Wager's observation of endokaryogamy in the basidium by the discovery of a nuclear fusion in the ascus similar to that in the basidium and teleutospore. Dangeard pronounced these nuclei which fuse in the ascus the morphological equivalents of the gametes of other fungi and algæ and the resulting ascus a fertilized egg; a view in which he has not been followed by other students of the group. It seems probable, however, that this fusion functionally may have the value in greater or less degree of a sexual fusion, and that in cases in which the normal union of gametes has disappeared this endokaryogamy may be a substitute for it.

The most puzzling feature of the sexual reproduction in the ascomycetes and that about whose existence, I may add, there is least agreement, lies in the fact that in the course of a single life cycle we have two nuclear fusions. At the origin of the ascocarp we find the formation and fusion of normally developed sexual cells and nuclei and in the young ascus a fusion of included nuclei. There is practically no dispute at present that the gametes formed at the origin of the ascocarp represent the original and normal sex organs of the fungus and for *Pyronema* the fusion between the antheridium and trichogyne is universally admitted.

My own studies have convinced me that in the mildews and *Pyronema* at least we have a normal conjugation of differentiated gametes at the origin of the ascocarp and an endokaryogamy in the ascus.

Blackman and Fraser confirm the existence of the two fusions in *Sphærotheca* and Blackman and Fraser (1906, *Humaria granulata*), Fraser and Chambers (1907, *Aspergillus Herbariorum*), Welsford (1907, *Ascobolus*), Fraser (1908, *Humaria rutilans*), Cutting (1909, *Ascophanus*) and Dale (1909, *Aspergillus repens*) describe the occurrence of two endokaryogamies in a single life cycle.

As already noted, Claussen in a recent preliminary paper attempts to resolve this difficulty in *Pyronema* by claiming that while there is a normal cell fusion at the origin of the ascocarp the nuclei of the gametes do not fuse, but are paired and divide by conjugate division in the ascogenous hyphæ, fusing only after they reach the young ascus. This is a direct transference to the ascomycetes of the conditions described by Blackman and Christman for the rusts, and it is certainly an obvious and easy suggestion. It was made in a letter to the writer by Raciborski in 1895 and later published by him.[1] In my opinion, as noted above, judging from the size of the nuclei in Claussen's figures of the young ascogenous hyphæ, the nuclear fusion has already occurred at this stage.

It is evident that such profound changes as have led to the wide-spread occurrence of endokaryogamy among so many and such diverse groups of the fungi can hardly have come about suddenly. Even on the mutation theory it would hardly be supposed that in the rusts, for example, the disappearance of function in the spermatia, the new fusion in the æcidium, the long series of conjugate divisions in the destructive uredo stage of the fungus and the endokaryogamy in the teleutospore should all have appeared by a single step simultaneously. There is a degree of correlation in all these changes without doubt, but they must fairly be assumed to have worked themselves out gradually in connection with the development of the complicated life history with heterœcism and the numerous spore forms which characterize the group.

It is evident that at present the fusion of nuclei in the teleutospore is simply the delayed union of the gametic nuclei that came together in the cell fusion at the base of the row of æcidiospores. In my opinion, however, as I have argued at length elsewhere, this fusion in the spore mother-cell originated as a purely vegetative union associated in the ascus and basidium with the development of the relatively gigantic size of these cells and the maintenance of the nucleo-cytoplasmic relation, the factors involved being the abundance of nutritive material concentrated in the ascus and basidium as sporebearing or-

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[2] *Flora,* 1896, p. 132.

gans and their function as spore mother-cells which are to undergo reduction divisions and close the sporophyte generation.

From this standpoint it is to be remembered that we have considerable evidence that in the rusts with abbreviated life cycles the binucleated condition and conjugate division extend back from the endokaryogamy to more or less vague and uncertain points in the life cycle of the sporophyte. For example, in the short-cycled rusts with only teleutospores Blackman, Maire and Olive find that the binucleated condition extends back from the teleutospore to some undetermined point in the mycelium from which the teleutospore sorus arises. As already noted, it is certain that in various basidiomycetes the binucleated condition extends back through the carpophore to some not sharply marked stage in the mycelium. Similar conditions may be present in the smuts.

In *Pyronema* I have found that the paired nuclei in the young ascus arise by simultaneous division of two mother nuclei in the tip of the ascogenous hypha from which an ascus is to arise and that the spindles are so placed that the two nuclei which are cut off in the young ascus and subsequently fuse are not sister nuclei. This final nuclear division is similar to the conjugate nuclear divisions in the rusts. Recently Guilliermond and Maire have described cases among certain of the larger discomycetes in which the ascogenous hyphæ were found to consist of binucleated cells for some distance below the young ascus, and suggest that these are comparable to the binucleated series of cells in the rusts. In the majority of cases so far described the ascogenous hyphæ are multinucleated as in *Pyronema* and *Ascobolus*. In the mildews the cells of the ascogon below the ascus are uninucleated and it does not appear that the nuclei which fuse in the ascus come from any such distinct lines of nuclear descent as do those which fuse in the teleutospore of the rusts.

The binucleated condition may be working backward from the endokaryogamy in the ascus in the forms noted by Guilliermond and Maire, and further we have no cases as yet in which the binucleated condition originating in a cell conjugation without nuclear fusion is working forward into the sporophyte. In the full-cycled rusts the binucleated condition and conjugate division extend from the gametic union to the reduction stage in the spore mother-cell. This is manifestly a condition of equilibrium comparable to the stable conditions present in most forms with definitely limited alternation of generations. In *Pyronema* in case Claussen's preliminary contentions should turn out to be true the same condition would be presented. We have nowhere evidence that the binucleated condition and conjugate division originated in gametic fusions and worked gradually forward through the sporophyte.

As perhaps the most striking evidence that the saprophytic habit has not led to the disappearance of sexuality we have the recent evidence as to the occurrence of sexual fusions in the yeasts. It is probably too early to speak with definiteness as to nuclear phenomena in these minute cells. Still there can be no question that in *Zygosaccharomyces* we have normal conjugation of gametes followed by two divisions and spore formation quite probably representing a reduction stage. In *Saccharomyces Ludwigii* just as clearly a double division period and endospore formation is followed by conjugation. This transposition of the sexual stage may be secondarily developed as Guilliermond believes, or may indicate the polyphyletic nature of the yeast group. There can at least be no question that sexuality either in a primitive or modified form has been maintained in this otherwise highly specialized saprophytic group.

Our knowledge of the conditions in the smuts as the result of the work of Dangeard, myself, Federley and Lutman, in a paper now in press, indicates that in this group also two fusions may occur in a single life cycle but under conditions which make the relations of the two to each other and to the original sexual reproduction of the group much clearer. In the smuts we have an endokaryogamy in the smut spore. In *Entyloma* the mycelium from which the spore arises is binucleated at least in the later stages of its development. In *Ustilago* the mycelial cells are multinucleated and there is less evidence for the separate ancestry of the nuclei in the smut spore.

The conjugations of the conidia regarded by DeBary as sexual unions represent the only normal conjugation of gametes in the life cycle, but they are plainly only of sporadic occurrence. Certainly they are not necessary to the completion of the normal development of the smut and they may probably be assumed to be in process of disappearance. The behavior of the nuclei in these fusing conidia favors this view. Federley finds them fusing in normal fashion in the smut of salsify. Lutman finds also that the nuclei may fuse in the conjugation of the promycelial cells of the oat smut. Dangeard finds they do not fuse in the conidia of *Tilletia* and according to my own observation the same is true in the conjugation of the conidia of the anther smut, though here many of the ordinary effects of sexual fusion appear in the conjugated pairs of conidia.

Lutman shows that infection and the normal development of oat smut may occur without any such fusions, and that hence the later nuclear fusion in the smut spore can not possibly be a delayed fusion of nuclei which came together in an earlier normal cell fusion of gametes. The endokaryogamy in the smut spore appears here as a distinct process which has originated independently of the normal sexual fusion, though it may have secondarily developed sexual significance with the development of conjugate division in the mycelial cells from which the smut spores arise, and have thus made possible the entire disappearance of the original normal sexual reproduction.

From this standpoint we are justified still further in the assumption that the appearance of binucleated cells and possibly conjugate division in the ascogenous cells of the discomycetes mentioned above indicate not the persistence of unfused but paired gametic nuclei, but a working back of conjugate division from the spore mother cell into the tissues of the sporophyte, thus giving gradually to the endokaryogamy in the ascus more and more of a sexual significance.

The evidence has accumulated in many lines that in several groups of the fungi the sexual process is followed by a longer or shorter period of development with cells containing a double chromosome number. Evidence from a direct counting of the chromosomes before and after fusion is lacking, owing to the small size of the nuclei, still there can be no reasonable doubt that the regularly binucleated cells of the rusts which form an unbroken series from the æcidium to the teleutospore contain twice the number of chromosomes present in the uninucleated mycelial cells from which the æcidium arises. As already noted, the conditions in the rusts are especially favorable for demonstrating that the sexual fusion inaugurates a period of development with cells containing 2n chromosomes and that this sporophytic stage tends to predominate in the life cycle both as to length and complexity.

It is quite evident also in advance of a knowledge of the complete cytological data that the double division involved in forming the promycelial cells is to be considered as a reduction period.

In the smuts and Basidiomycetes the limits of the gametophyte and sporophyte are not so definitely marked as in the rusts, but the binucleated phase is certainly to be regarded as sporophytic however it may be inaugurated. In the Ascomycetes the morphological relations of the mycelium and ascocarp have long been regarded as showing a parallelism with the conditions in liverworts and mosses. The fact of a triple division in the ascus in contrast with the elsewhere universally present double division of spore mother cells has been regarded as opposed to the interpretation of the ascus as a spore mother cell. However, as I have pointed out, this triple division replacing ordinary double division is to be regarded as correlated with two nuclear fusions preceding it.

Miss Fraser has brought further interesting cytological evidence that the third division in the ascus is a reduction division which she characterizes as a brachymeiosis. The universality of the triple nuclear division in the ascus even in cases where less than eight spores are formed is certainly to be regarded as a fact of the first importance and comparable to the universality with which a double division occurs in other spore mother cells. The most striking of the peculiarities as to cell and nuclear fusions in the fungi may be summarized as follows:

1. The fusion of multinucleated gametes.
2. The male element may be a mass of gonoplasm rather than a definitely bounded cell.
3. Endokaryogamy, the fusion of nuclei not brought together by cell fusion but of more or less independent ancestry.
4. The fusion of gametes without the fusion of their nuclei; the latter reproducing by conjugate division for long series of cell generations and finally fusing just before the reduction division.
5. Fertilization by nuclear migration from a vegetative cell to an egg or fertile cell.
6. Two successive fusions in the same life cycle, a normal conjugation of gametes and later endokaryogamy.